INFLUENCE OF WATER MOVEMENT AND ROOT GROWTH ON THE DOWNWARD DISPERSION OF *ROTYLENCHULUS RENIFORMIS*

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ABSTRACT

Moore, S. R., K. S. Lawrence, F. J. Arriaga, C. H. Burmester, and E. van Santen. 2011. Influence of Water Movement and Root Growth on the Downward Dispersion of *Rotylenchulus reniformis*. Nematropica 41:75-81.

The presence of *Rotylenchulus reniformis* below the plow layer can have negative effects on cotton production by restricting the uptake of water and nutrients by roots in the lower soil horizons. Two trials were established in 7.62-cm diameter by 75-cm deep soil cores to determine 1) the effect of water movement on vertical translocation of *R. reniformis*, and 2) the role of root growth in the downward migration of *R. reniformis*. The water movement study consisted of three treatments of simulated rainfall amounts, 25-mm, 76-mm, and 127-mm, and no rainfall. Water movement minimally affected the movement of *R. reniformis* through the soil profile. Nematodes were observed to a depth of 30-cm after the 25-mm rain event. *Rotylenchulus reniformis* was detected to a depth of 45-cm following 76-mm of rainfall and to the maximum sampling depth of 75-cm following 127-mm of rainfall. Cotton roots reached the maximum depth of 75-cm at 60 days after planting (DAP). Vermiform life stages reached 75-cm at 45 DAP. Females colonized roots to a depth of 62-cm at 90 DAP. Cotton roots were observed to exhibit less growth when the numbers of females embedded in the roots was the highest.

Key Words: behavior, cotton, root growth, *Rotylenchulus reniformis*.

RESUMEN

Moore, S. R., K. S. Lawrence, F. J. Arriaga, C. H. Burmester, and E. van Santen. 2011. Influencia del movimiento del agua y el crecimiento de las raíces sobre la dispersión vertical de *Rotylenchulus reniformis*. Nematropica 41:75-81.

La presencia de *Rotylenchulus reniformis* por debajo de la capa arable puede tener consecuencias negativas sobre la producción de algodón, al restringir la toma de agua y nutrientes en los horizontes inferiores del suelo. Se establecieron dos experimentos en secciones de suelo de 7.62 cm de diámetro y 75 cm de profundidad para deteminar 1) el efecto del movimiento del agua sobre la translocación vertical de *R. reniformis*, y 2) el papel del crecimiento de las raíces en la migración vertical de *R. reniformis*. El estudio del movimiento del agua consistió de tres tratamientos de cantidad de lluvia simulada, 25-mm, 76-mm, y 127-mm, y un control sin lluvia. El movimiento del agua afectó el movimiento de *R. reniformis* de manera mínima a través del perfil del suelo. Se observaron nematodos hasta una profundidad de 30 cm después de una lluvia de 25 mm. Se detectó el nematodo hasta una profundidad de 45 cm después de una lluvia de 76 mm, y hasta la profundidad máxima de muestreo (75 cm) después de una lluvia de 127 mm. La raíces de la planta de algodón alcanzaron una profundidad máxima de 75 cm a los 60 días después de la siembra (DDS). Los estados vermiformes se encontraron a 75 cm de profundidad a los 45 DDS. Las hembras colonizaron raíces hasta una profundidad de 62 cm a los 90 DDS. Se observó menor crecimeinto de las raíces de la planta de algodón cuando el número de hembras en las raíces fue más alto.

Palabras clave: algodón, comportamiento, crecimiento de raíces, *Rotylenchulus reniformis*

INTRODUCTION

Rotylenchulus reniformis (Linford and Oliveira) has become the most economically damaging pathogen in Alabama, causing an average of 7% yield loss totaling nearly $126 million over the past decade (Blasingame *et al*., 2009). Yield losses can reach levels as high as 40% and are caused by females feeding on the roots, restricting the flow of water and nutrients to the plant (Robinson, 2007). In a manuscript currently in press (Moore *et al*., 2011) *R. reniformis* was introduced into the upper 15-cm of a cotton field previously absent of the nematode and was observed to colonize the root zone to depths below the plow layer within the first growing season and survive until the following year. Survival of *R. reniformis* at depths well below the plow layer can directly affect cotton yields (Robinson *et al*., 2005) and enable rapid population resurgence into the upper horizons subsequent to rotation or nematicide application (Lee *et al*., 2002; Newman and Stebbins, 2002). Although the existence and consequences of deep populations of *R. reniformis* are well documented, the factors that influence these populations are unknown. The aim of this research is to explore two possible factors that influence downward dispersion of *R. reniformis*: water movement and root growth.

MATERIALS AND METHODS

Two trials to monitor the migration of *R. reniformis* through the soil profile, the first as affected by water movement and the second as affected by root growth, were established at the Auburn University Plant Science Research Center in Auburn, AL. The trials were conducted in soil cores, 7.62-cm in diameter and 75-cm deep, collected at the Tennessee Valley Research and Extension Center, near Belle Mina, AL using a #5-UV4 Model GSRPSUV4G ATV mounted soil core sampler (Giddings Machine Company, Windsor, CO). The soil is classified as a Decatur silt loam (fine, kaolinitic, thermic, Rhodic Paleudults: 23%, 49%, 28%, sand-silt-clay, 1% OM, pH 6.2) and was tested to confirm the absence of R. reniformis at all depths. This same soil type was utilized by Moore *et al.* (2011) for a study of in-field movement of *R. reniformis* and was chosen to compliment the findings presented there. The soil cores were supported in wooden racks and nylon wicks were attached to the bottom of each to facilitate water drainage. The soil cores were visually examined to confirm absence of soil compaction or other disturbances caused by collection and blocked by similarities in depth of horizon distribution. A week prior to initiation of the trials, the soil cores moisture was brought to field capacity and allowed to acclimate to their environment.

The *R. reniformis* nematodes were obtained from stock cultures grown on cotton (*Gossypium hirsutum* L.), cv. Delta and Pine Land (DPL) 555 BGRR at Auburn University Plant Science Research Center. The nematodes were increased in 10-cm diameter polystyrene pots containing 500-cm^3 of a loamy sand soil (72.5%, 25%, 2.5%, sand-silt-clay, 1% OM, pH 6.4). The soil was autoclaved at 121°C and 103.4 kPa for 2 hours on two successive days for sterilization. Nematode inoculum consisted of *R. reniformis* vermiform life stages extracted from the soil using combined gravity screening and sucrose centrifugal flotation (Jenkins, 1964).

Water Movement: Rotylenchulus reniformis was added to the top 2.5-cm of soil by pipetting in 50,000 vermiform life stages (juveniles and adults) in 2-ml of water and were allowed to acclimate for 12 hours. The cores were then subjected to one of three different simulated rainfall amounts: 25-mm, 76-mm, and 127-mm using a drip rainfall simulator, or no rainfall simulating a dry control. The drip rainfall simulator consisted of a rubber-capped plastic reservoir with five, 200-µl pipette tips that applied approximately 25-mm of water per minute. The trial was arranged in a randomized complete block design with six replicates and repeated for a total of twelve replicates. Forty-eight hours after the simulated rain event cores were separated by cutting into 15-cm sections (0 – 15–cm, 15 – 30–cm, 30 – 45–cm, 45 – 60–cm, and 60 – 75– cm). The nematodes were extracted from each soil section and enumerated.

Root Growth: One cotton plant, (DPL 555 BGRR) was planted in each soil core and *R. reniformis* was added to the top 2.5-cm of soil by pipetting in 10,000 vermiform life stages in 2-mL of water. The cotton was then allowed to grow until termination 15, 30, 45, 60, 75, or 90 days after planting. Planted soil cores were placed in the greenhouse at an average temperature of 30°C and watered as needed. Plants were fertilized weekly using Peter's 20-10-20 water-soluble fertilizer (The Scott's Company, Marysville, OH). Soil cores were arranged in a randomized complete block design and replicated three times. At each respective termination date, the cores were divided into 15-cm sections for analysis. The cotton roots were carefully removed by washing from each section, weighed, and stained with acid fuchsin as described in Byrd *et al*., (1983). The stained roots were evaluated for numbers of embedded females using a Nikon SMZ800 stereo microscope at 63x magnification. The soil from each section was evaluated for number of vermiform life stages that were extracted and enumerated as previously described. All tests were conducted twice, and data collected were analyzed by SAS (SAS Institute, Inc) using generalized linear models. Means were compared by Dunnett's Test, with the 0 to 15-cm depth as the reference group.

RESULTS

Water Movement: Water movement minimally affected the dispersion of *R. reniformis* in our trials (Table 1). Minimal independent migration of *R. reniformis* was observed as no nematodes were detected below the top 15-cm within the 0-mm treatment. However as the amount of rainfall increased, so too did the depths at which *R. reniformis* was detected. Nematodes were detected to a depth of 30-cm when 25-mm of rainfall

Table 1. Percent of *R. reniformis* population recovered from sampling depths and simulated rainfall amounts.

	Depth = 0 - 15 cm			Depth = 16 - 30 cm		
Rainfall (mm)	Mean[z]	95% CL	Dunnett's *P*[y]	Mean[z]	95% CL	Dunnett's *P*[y]
0	100	(99.9, 100.0)		0	(0.0, 0.1)	
25.4	94.2	(87.6, 97.4)	<0.0001	5.8	(2.6, 12.5)	<0.0001
76.2	98.4	(94.9, 99.5)	<0.0001	1.4	(0.4, 4.3)	<0.0001
127	93.1	(85.5, 96.9)	<0.0001	2.2	(1.0, 5.0)	<0.0001
	Depth = 31 - 45 cm			Depth = 46 - 60 cm		
Rainfall (mm)	Mean[z]	95% CL	Dunnett's *P*[y]	Mean[z]	95% CL	Dunnett's *P*[y]
0	0	(0.0, 0.1)		0	(0.0, 0.1)	
25.4	0	(0.0, 0.0)	0.9982	0	(0.0, 0.0)	0.9986
76.2	0.2	(0.1, 0.8)	0.0119	0	(0.0, 0.0)	0.751
127	1.7	(0.7, 4.0)	<0.0001	2.2	(1.0, 5.0)	<0.0001
	Depth = 61 - 75 cm					
Rainfall (mm)	Mean[z]	95% CL	Dunnett's *P*[y]			
0	0	(0.0, 0.1)				
25.4	0	(0.0, 0.0)	0.9986			
76.2	0	(0.0, 0.0)	0.7477			
127	0.3	(0.1, 0.9)	0.0016			

[z]Means calculated as percent of recovered population.

[y]Probability of being different from the control (0-mm rainfall).

was applied, to a depth of 45-cm in the 76-mm rainfall treatment, and to the maximum sampling depth of 75-cm when 127-mm of rainfall was applied.

Root Growth: The numbers of *R. reniformis* females embedded in the roots remained largely in the upper 15-cm of the root zone for the majority of the trial (Table 2). Detectable levels of embedded females were observed within the top 30-cm at 15 and 30 days after planting (DAP). From 45 to 75 DAP embedded females were observed in the upper 45-cm of the root zone and were detected within the 60 – 75–cm depth only at 90 DAP. Significantly larger numbers, between 95.5 and 99% of all observed embedded females, were found in the upper 15-cm of the root zone from 15 to 75 DAP. At 90 DAP the number of embedded females in the 16 – 30-cm depth increased to 25.8% of the total and was not significantly different from the top 15-cm.

Similarly, the numbers of *R. reniformis* vermiform life stages in the soil remained largely in the upper 15-cm (Table 3). However detectable levels of vermiform life stages were found to the maximum sampling depth of 75-cm at 30 DAP and throughout the remainder of the trial. Greater than 80% of all recovered vermiform life stages were found in the top 15-cm of the root zone from 15 to 75 DAP. Levels in the 16- to 30-cm depth increased to 32.1% of recovered vermiform life stages between 75 and 90 DAP and were not significantly different from the upper 15-cm.

The number of *R. reniformis* females per gram of root fluctuated at fifteen day intervals throughout the trial (Table 4). At 15, 45, and 75 DAP, the number of females per gram of root were significantly higher within the top 15-cm of the root zone compared to all other depths. However, the ratio of females per gram of root in the top 15-cm declined sharply at 30, 60, and 90 DAP compared to the previous observation. Total root mass and numbers of *R. reniformis* females embedded within cotton roots exhibited sinusoidal wave patterns 180° out of phase (Figure 1). The increase in cotton root mass in 15 day intervals was the smallest when the

Table 2. Percent of total *R. reniformis* females embedded in the cotton roots at each sampling depth by sampling date.

	DAP[z] = 15			DAP = 30		
Depth (cm)	Mean[y]	95% CL	Dunnett's *P*[x]	Mean	95% CL	Dunnett's *P*
0 - 15	98.7	(77.4, 99.9)		95.5	(71.4, 99.4)	
16 - 30	1.2	(0.1, 19.9)	0.0003	3.7	(0.5, 24.9)	<0.0001
31 - 45	0.0	(0.0, 2.4)	0.0003	0.0	(0.0, 1.9)	0.0002
46 - 60	0.0	(0.0, 2.4)	0.0003	0.0	(0.0, 1.9)	0.0002
61 - 75	0.0	(0.0, 2.4)	0.0003	0.0	(0.0, 1.9)	0.0002
	DAP = 45			DAP = 60		
Depth (cm)	Mean	95% CL	Dunnett's *P*	Mean[z]	95% CL	Dunnett's *P*
0 - 15	98.9	(90.3, 99.9)		96.9	(74.6, 99.7)	
16 - 30	0.4	(0.0, 4.4)	<0.0001	2.7	(0.3, 23.1)	<0.0001
31 - 45	0.1	(0.0, 0.7)	<0.0001	0.1	(0.0, 0.4)	<0.0001
46 - 60	0.0	(0.0, 0.2)	<0.0001	0.0	(0.0, 0.6)	0.0001
61 - 75	0.0	(0.0, 0.2)	<0.0001	0.0	(0.0, 0.6)	0.0001
	DAP = 75			DAP = 90		
Depth (cm)	Mean	95% CL	Dunnett's *P*	Mean	95% CL	Dunnett's *P*
0 - 15	98.3	(86.2, 99.8)		73.7	(21.4, 96.7)	
16 - 30	2.4	(0.3, 17.4)	<0.0001	25.8	(3.0, 79.4)	0.3506
31 - 45	0.2	(0.0, 2.0)	<0.0001	4.3	(0.5, 27.7)	0.0030
46 - 60	0.0	(0.0, 0.1)	<0.0001	0.6	(0.0, 75.4)	<0.0001
61 - 75	0.0	(0.0, 1.0)	<0.0001	0.1	(0.0, 1.0)	<0.0001

[z]Days after planting.
[y]Means calculated as percent of recovered population.
[x]Probability of being significantly different from the control (0 – 15-cm depth)

increase in numbers of *R. reniformis* females embedded in the roots in 15 day intervals was the largest.

DISCUSSION

The availability of a food source is the apparent drive behind colonization of the soil horizon by *R. reniformis*. As the cotton roots elongated and extended into the soil, so too did the nematode populations. Newman and Stebbins (2002) and Lee *et al.*, (2002) both observed a late season resurgence of *R. reniformis* populations from the lower horizons to the upper horizons following either an early season nematicide treatment or rotation to a non-host, such as corn. In their trials, the food source in the upper horizons was diminished or deprived for a period, but once a food source became available, *R. reniformis* populations were again able to colonize the upper soil horizons.

The influence of water movement on the downward dispersal of *R. reniformis* was minimal in our trial. A large amount of rainfall was required to transport even small amounts of *R. reniformis* downward from the top 15-cm of the profile. However, the significance of even small populations of *R. reniformis* in the lower horizons, where root mass is less but critical to plant health, can be great (Browning *et al.*, 1975; Taylor and Klepper, 1978). *Rotylenchulus reniformis* introduced later in the season, possibly on contaminated equipment during a side dress application of a fertilizer or nematicide, could find roots in the lower horizons and thus could become a significant yield-limiting factor if able to establish there. Robinson *et al.*, (2005) found that populations of *R. reniformis* below the plow layer can suppress root growth and decrease the ability to supply moisture to the plant. This can be an even greater factor in dry years when moisture is unavailable in the upper horizons. Furthermore, control of *R. reniformis* at these depths is only experimental and expensive. As

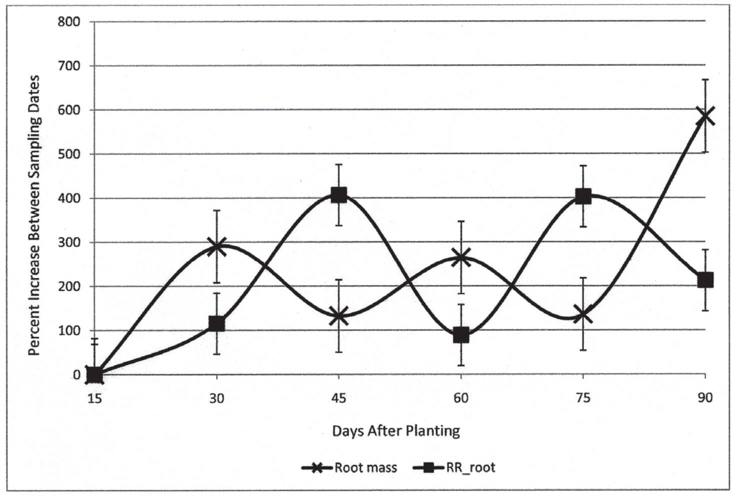

Fig. 1. Percent increase between sampling dates of the average root mass and *R. reniformis* females embedded in cotton root (RR_root) by sampling date. Each value is an average of 6 replicates.

Table 3. Percent of recovered *R. reniformis* vermiform life stages at each sampling depth by sampling date.

	DAP[z] = 15			DAP = 30		
Depth (cm)	Mean[y]	95% CL	Dunnett's *P*[x]	Mean	95% CL	Dunnett's *P*
0 - 15	90.3	(75.8, 96.5)		80.4	(39.2, 96.3)	
16 - 30	8.8	(3.0, 22.5)	<0.0001	9.3	(2.2, 32.3)	0.0045
31 - 45	0.0	(0.0, 0.1)	<0.0001	5.0	(0.8, 26.1)	0.0028
46 - 60	0.0	(0.0, 0.1)	<0.0001	2.6	(0.8, 7.9)	<0.0001
61 - 75	0.0	(0.0, 0.1)	<0.0001	1.2	(0.3, 4.6)	<0.0001
	DAP = 45			DAP = 60		
Depth (cm)	Mean	95% CL	Dunnett's *P*	Mean[z]	95% CL	Dunnett's *P*
0 - 15	91.6	(68.2, 98.2)		92.0	(65.6, 98.6)	
16 - 30	4.8	(1.1, 18.3)	<0.0001	3.9	(0.7, 18.4)	<0.0001
31 - 45	2.5	(0.5, 11.5)	<0.0001	3.3	(0.5, 18.2)	<0.0001
46 - 60	1.5	(0.3, 7.8)	<0.0001	0.0	(0.0, 0.0)	<0.0001
61 - 75	0.2	(0.1, 1.0)	<0.0001	0.3	(0.1, 1.0)	<0.0001
	DAP = 75			DAP = 90		
Depth (cm)	Mean	95% CL	Dunnett's *P*	Mean	95% CL	Dunnett's *P*
0 - 15	92.1	(64.4, 98.7)		60.3	(20.1, 90.1)	
16 - 30	5.6	(0.8, 31.4)	0.0006	32.1	(7.0, 74.7)	0.6739
31 - 45	0.4	(0.1, 1.9)	<0.0001	4.5	(1.4, 13.9)	0.0024
46 - 60	0.3	(0.0, 1.6)	<0.0001	0.4	(0.1, 1.9)	<0.0001
61 - 75	0.9	(0.2, 5.1)	<0.0001	0.2	(0.0, 0.8)	<0.0001

[z]Days after planting.
[y]Means calculated as percent of recovered population.
[x]Probability of being significantly different from the control (0 – 15-cm depth).

Table 4. Number of *R. reniformis* females per gram of root at each sampling depth by sampling date.

	DAP[z] = 15			DAP = 30		
Depth (cm)	Mean[y]	95% CL	Dunnett's *P*[x]	Mean	95% CL	Dunnett's *P*
0 - 15	860.2	(447.5, 1272.9)		396.6	(0.0, 809.3)	
16 - 30	70.0	(0.0, 482.7)	0.0243	74.6	(0.0, 487.3)	0.6088
31 - 45	0.0	(0.0, 0.0)	0.0126	0.0	(0.0, 0.0)	0.4301
46 - 60	0.0	(0.0, 0.0)	0.0126	0.0	(0.0, 0.0)	0.4301
61 - 75	0.0	(0.0, 0.0)	0.0126	0.0	(0.0, 0.0)	0.4301
	DAP = 45			DAP = 60		
Depth (cm)	Mean	95% CL	Dunnett's *P*	Mean[z]	95% CL	Dunnett's *P*
0 - 15	1150.3	(737.3, 1562.8)		600.0	(187.3, 1012.7)	
16 - 30	76.2	(0.0, 488.9)	0.0014	100.3	(0.0, 513.0)	0.2365
31 - 45	20.8	(0.0, 433.5)	0.0008	22.2	(0.0, 434.9)	0.1388
46 - 60	0.0	(0.0, 0.0)	0.0006	0.0	(0.0, 0.0)	0.1180
61 - 75	0.0	(0.0, 0.0)	0.0006	0.0	(0.0, 0.0)	0.1180
	DAP = 75			DAP = 90		
Depth (cm)	Mean	95% CL	Dunnett's *P*	Mean	95% CL	Dunnett's *P*
0 - 15	1456.7	(1044.0, 1869.4)		353.3	(0.0, 766.0)	
16 - 30	113.0	(0.0, 525.7)	< 0.0001	283.4	(0.0, 696.1)	0.9973
31 - 45	16.6	(0.0, 429.32)	< 0.0001	123.4	(0.0, 536.1)	0.8279
46 - 60	0.0	(0.0, 0.0)	< 0.0001	36.7	(0.0, 449.4)	0.6223
61 - 75	0.0	(0.0, 0.0)	< 0.0001	6.7	(0.0, 419.5)	0.5478

[z]Days after planting.

[y]Means calculated as percent of recovered population.

[x]Values are significantly different from the control (0 – 15-cm depth) where $P \leq 0.05$.

such, the introduction of *Rotylenchulus reniformis* into the surface of a cotton field can have much more dire consequences.

The analysis of root growth rates and *R. reniformis* population fluctuations produced interestingly inverse wave patterns. Although not compared with root systems absent of nematodes, these inverse wave patterns could grant further insight to the nematode/root relationship that occurs during the growing season. Determination of the presence or absence of patterns such as this in the field could be used to predict management opportunities, especially for mid-season nematicide applications. Additional benefits of the knowledge of these patterns could be gained in the form of strategic timing of irrigation or fertilizer applications to aid the cotton plant when root growth restriction is at its greatest. More knowledge of the interactions of *R. reniformis* and cotton root growth may be utilized to allow for more efficient management of this pathogen.

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Received:
Recibido: *25/I/2011*

Accepted for publication:
Aceptado para publicación: *1/III/2011*